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FOR

LATERAL FLOW ASSAY DEVICES AND METHODS FOR CONDUCTING ASSAYS

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Background of the Invention

Membrane-based test devices, particularly devices used in diagnostic medicine, employ a variety of internal and external calibrators to provide a qualitative or a quantitative result for an analyte of interest in a test solution. One type of membrane-based test device is a lateral flow assay.

In general, lateral flow assays are membrane-based test devices in which a sample that is suspected of containing the analyte of interest is placed at or near one end of a membrane strip. The sample is carried to the opposite end of the membrane strip by a liquid phase that traverses the membrane strip by capillary action. While traversing the membrane strip, the analyte in the test sample, if any, encounters one or more "capture" reagents with which it may react to produce a detectable signal.

The early types of immunochromatography devices, such as those taught in United States Patent No. 4,366,241 (Tom et al.), lacked an internal reference. Later devices, such as taught in United States Patent No. 4,374,925 (Litman) do indeed employ an internal reference. In some instances, the internal references used have required fairly laborious cross-referencing to achieve results.

One known membrane-based test device is the dipstick. The dipstick is a stick having a small reagent impregnated membrane

(stripped with capture reagents on different zones and wicking pads on one end and the other) end for dipping into a test solution either containing or suspected of containing the analyte of interest. The dipstick membrane (hereinafter "dipstick") develops a color that is proportional to the concentration of the analyte of interest in the test sample. Typically, the user determines the concentration of the analyte by comparing the color on the membrane to the color on an external calibration, such as a series of colored plates that are printed on a label. This is a subjective determination.

External calibration of a dipstick, via colored plates, provides several problems. First, it is difficult to accurately match the color of the plates with the color on the dipstick. Secondly, the color on the plates would not fade in proportion to the adverse conditions affecting the color on the dipstick. Further, the color on the plates would at best only be accurate for a particular set of reaction conditions.

Many of such devices and methods rely upon calibration to provide valid and meaningful results for semi-quantitative and quantitative detections. Calibration methods are often critical to provide accurate, reliable and reproducible results, especially when the environments and conditions under which the measurements are commenced are not carefully controlled. Two calibration methods, external and internal calibrations, are commonly employed. In the external calibration method, a standard curve is usually obtained from

standard samples containing a series of a known amount of analyte, and the results obtained from the samples are then compared with the standard curve to extract the information regarding the presence and/or amount of the analyte in the sample. External calibration methods are often subject to interference from environmental and batch-to-batch variations, and sometimes are not reliable. When an instrument or measuring device is used, it is also subject to interference from the instability of the instrument or device.

In general, lateral flow assay methods are limited in their sensitivity by not using an internal calibration mechanism that takes into account widely varying differences in temperature, flow conditions, capillary action, pressure, and other factors that affect movement or deposition of analyte on a membrane or support. Any system that compares the migration characteristics of analyte on a given test strip with references taken on another separate strip at another time and place will not achieve maximum sensitivity and accuracy.

What is needed in the industry is a lateral flow assay system and method having improved sensitivity in relation to existing methods and devices. The invention of this application is directed to such an application.

Summary of the Invention

A lateral flow assay system, apparatus, and method are provided in the invention. The assay provides a method to detect the quantity of

analyte residing in a test solution. The assay further comprises a probe. The probe may be of various types. Probes are configured for generating a detectable signal. Probes may be covalently reacted with antibody to form probe-conjugates and this conjugate then may travel to react with analyte to form a probe-conjugate analyte complex (or "sandwich"). Once this species is immobilized upon a detection zone it is referred to as a "sandwich complex". All these described species may be able to migrate on a membrane and may be used for analyte detection.

A membrane may be configured to provide a sample pad, conjugate pad, detection zone, calibration zone and wicking pad. On the conjugate pad the probes and probe-conjugates may be dried down upon the membrane and made available for analyte as the analyte moves along the membrane from one end of the membrane to the other end. When analyte molecules join probe conjugates, they form probe conjugate analyte complexes, which are capable of becoming mobilized, and moving to a detection zone.

Upon the detection zone, a first capture reagent may be immobilized. The first capture reagent may be composed of any ligand specific binder, thus, one example of such a capture reagent is an antibody. This first capture reagent may immobilize such probe-conjugate analyte complexes to form a "sandwich complex", or "sandwich" upon the detection zone.

A calibration zone also may be provided. The calibration zone comprises at least two control lines, however, in some applications of the invention, three, four, or more control lines may be provided. The control lines may have applied thereon a predetermined amount of a second capture reagent. The second capture reagent may be configured to immobilize probe-conjugates or probes that migrate to the control lines without analyte, thereby positioning them for generating a calibration or control signal.

In general, each control line may have a predetermined amount of the second capture reagent. Thus, commonly, the first control line nearest the detection zone may have the least amount of second capture reagent, while the last control line furthest from the detection zone may have the greatest amount of second capture reagent. In some applications, the control lines may vary by predetermined amount from each other, so as supply a suitable calibration curve, as shown in herein and described with reference to Figure **2**.

Some applications of the invention utilize visual comparisons. In other applications, reading devices such as reflectometer or spectrophotometer may be employed to compare the intensity of signals generated with reference standards that are generated in the assay. Spectrophotometric methods may be employed to compare the intensity of signals generated with reference standards that are generated in the course of the assay.

In some applications, a calibration data curve may be generated using signal intensity data generated from the control lines. The curve may provide a "look-up table" that may be automatically applied in an algorithm of an analytical instrument.

The probe in some cases may comprise a microparticle that is capable of generating a visual signal, such as a latex bead, for example, that includes red or blue or another colored dye. In other applications, the probe may generate fluorescent signals that are detectable and are proportional to the amount of such species in a given zone.

The invention may be directed to a method for detecting the quantity of an analyte in a test solution. The method may include applying a plurality of probes that are configured for migrating to preselected locations and, when they become sandwich complexes, generating a detectable signal.

Internal calibration methods are useful because such methods may provide more accurate, more reliable and more reproducible results than external calibration methods. Using internal calibration methods, signals related to the analyte in the sample are usually measured at the same time and/or upon the same membrane device that generates the calibration signals. The simultaneous measurements can eliminate some potential interference to provide more consistent and sensitive detections.

Brief Description of the Drawings

A full and enabling disclosure of this invention, including the best mode shown to one of ordinary skill in the art, is set forth in this specification. The following Figures illustrate the invention:

Figure **1** is a top view of one embodiment of the invention, showing a lateral flow assay having three control lines in a calibration zone;

Figure **1A** shows a perspective schematic view of the movement of fluids and the formation of complexes upon the surface of the membrane strip of a lateral flow assay, showing the membrane strip after a test sample containing analyte has been applied to the sample pad,

Figure **1B** shows the same schematic view of the membrane test strip shown in Figure **1A**, but at a later time after migration of fluids have occurred and complexes have formed; and

Figure **2** shows a calibration curve that may be used in some applications of the invention.

Detailed Description of the Invention

Reference now will be made to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not as a limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in this invention without departing from the scope or spirit of the invention.

The invention makes it possible to use multiple control lines to quantify analytes of interest in a lateral flow assay format. In particular, the method and apparatus of the invention relate to conducting internal calibrations by: (1) quantifying the analyte and (2) calibrating the assay device, at about the same time, on the same membrane device. That is, calibration and sample testing may occur on the same device, by affording a built-in calibration data curve generated using the testing device. A multi-point calibration technique may be employed in a lateral flow assay format.

The method may be used for quantitative and semi-quantitative detection. The probes used may reveal color intensity, fluorescence intensity, as examples. The probes for control lines may be microparticles such as latex beads, for example, labeled with essentially any signal generating species. Alternately, the probes may comprise labeled latex beads further conjugated with antibodies, as further described herein. The antibodies may be dried upon the conjugate zone of the membrane.

Various amounts of predetermined capture reagents may be provided on solid substrates, such as porous membranes, to form multiple control lines for calibration purposes. In yet another embodiment, the capture reagents may be antibodies. In yet another embodiment, the capture reagents may be any molecules which are

capable of forming strong interactions with probes and/or probe conjugates.

The membrane-based device of the invention comprises several components, including a membrane, a sample pad, a conjugate pad and a wicking pad, or a combination of these items. The membrane typically includes at least two zones, that is, a detection zone and a control zone. A sample pad contacts one end of the conjugate pad.

One design of the assay device includes a liquid sample flow direction through a sample pad, conjugate pad, detection zone of the membrane, control zone of the membrane, and wicking pad. In general, the wicking pad assists in promoting capillary action and fluid flow one-way through the membrane of the device, and the wicking pad "pulls" the liquid containing the analyte along the membrane from one end of the membrane to another end of the membrane.

Turning now to Figure **1**, a lateral flow assay **20** is provided in top view. The lateral flow assay **20** comprises a membrane **23** as a solid support, and includes a sample pad **21**. The sample pad **21** is configured to receive a liquid sample containing analyte **40** (seen in Figure **1A**). A conjugate pad **22** is provided further "downstream" of capillary movement direction **29**, as shown by the arrow on the left side of the Figure **1**.

Conjugate pad **22** typically contains probes **41** and probe conjugates **42** (see Figure **1A**) in a form that makes the probe

conjugates available for bonding with the analyte **40** as the analyte **40** passes from the sample pad **21**. A typical method employs microparticles as probes **41**, and their conjugate deposited on the conjugate pad **22**. Such particles may be comprised of latex, or other suitable material, as further described herein. Latex microparticles, when used as probes, may be colored with dyes that are visible to the eye, or to detection apparatus. Sometimes a probe **41** emits light (as in the case of fluorescence methods), or the probe **41** may be detected by other techniques once it has migrated and complexed, as further described herein.

A detection zone **31** is shown in Figure **1**. The detection zone **31** may comprise an immobilized capture reagent along detection line **24**, as further described in connection with Figure **1A**. A calibration zone **32** is shown with three control lines **25-27**. A wicking pad **28** also is shown.

Referring to Figure **1A**, a membrane **23** is provided in which molecules of the analyte **40** to be detected have been deposited upon the sample pad **21**. The analyte **40**, which is fluidized, moves in the direction of the arrow shown in Figure **1A** from one end of the membrane to the other.

Figure **1A** shows a schematic view in which the components of the assay **20** are enlarged for purposes of explanation. Figure **1A** shows membrane **23** at a point when the test sample or test solution has been applied to the sample pad **21** for only a short period of time.

Probes **41** are seen upon the conjugate pad **22**. Typically, probes **41** are dried or immobilized upon the conjugate pad **22**. Probe conjugate **42** also is immobilized upon the conjugate pad **22**. Once molecules of analyte **40** bind with probe conjugates **41-42**, they become probe conjugate analyte complexes (such as probe analyte conjugate complex **49-50** shown in Figure **1B**) which are mobile along the membrane **23**.

The detection zone **24** is shown in Figure **1A** having several capture reagents **43a-c** immobilized upon the detection zone **44**. These capture reagents **43a-c** serve as stationary binding sites for the probe analyte conjugate complexes **49-50** which migrate to them, as further shown in Figure **1B**. The chemical identity of capture reagents **43a-c** is further described herein.

The calibration zone **32** is shown near the end of the membrane **23**. The calibration zone **32** provides at least two or more control lines, shown in this particular example as control lines **25-27**. In many cases, the control lines are provided with a “binder” which is used to bind probe **41** molecules which pass the length of the membrane **23**. The "binder" may include an antibody, such as second antibody **47a-c** shown in Figure **1B**. The control lines **25-27** have a certain and specific amount of second antibody **47a-c** provided thereon, so that in a saturated environment having large amounts of probe **41** or probe conjugate **42**, they will reveal a specific, exact, and predetermined level of signal intensity. It will be recognized that thousands of molecules are provided

upon the membrane **23**, but the Figures **1A-1B** show only a few molecules, for purposes of illustration.

Figure **1B** shows the membrane **23** of Figure **1A** at a later point in time after the solution has migrated as shown in the arrow of Figure **1B**. A probe conjugate complex **49** and a probe conjugate complex **50** may be seen migrating from the conjugate pad **22** to the detection zone **44**. Several sandwich complexes **45a**, **b** and **c** have formed by the union of probe conjugate complexes similar to that shown as probe conjugate complex **49** with capture reagent **43a-c** (Figure **1A**), forming an immobilized sandwich complex **45a-c** within the detection zone.

Probes **41** and probe conjugates **42** which are not bound to analyte, also become mobile through the detection line **24** (see for example probe **52**), and continue beyond the detection line **24** to the calibration zone **32**. The calibration zone **32** includes calibration lines **25**, **26**, and **27**. The calibration lines **25-27** may be pre-loaded upon the membrane **23** with a second capture reagent, such as second antibody **47**, and thus an intensity of color is generated upon the calibration lines **25-27** upon migration of probe **41** or probe conjugates **42**. A control probe complex **56** may be formed when a probe **41** attaches. Likewise, a control probe conjugate complex **57** may be formed by attachment of a probe conjugate **42**. Both probes **41** and probe conjugates **42** are available for binding in the detection zone **32**.

An excess of probe molecules, such as dyed microparticles, can be employed in the assay **20**, so that each calibration line **25-27** reaches its full and predetermined potential for signal intensity. That is, the amount of probe **41** molecules that are deposited upon calibration lines **25-27** are predetermined because the amount of capture reagent employed on the calibration lines **25-27** is set at a predetermined and known level. A comparison may be made between the intensity levels of the calibration lines **25-27** and the detection line **24** to calculate the amount of analyte **40** present in the sample or solution. This comparison step may occur visually, or with the aid of a reading device (not shown). Wicking pad **28** receives the fluid that has migrated through membrane **23**.

The membrane **23**, or solid support, which is employed in the assay may be a cellulose ester. Nitrocellulose is known provides good results in some applications. It should be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, in particular, aliphatic carboxylic acids having from one to seven carbon atoms.

Although nitrocellulose may be a suitable material for producing the membrane, it is to be understood that other materials may also be employed for such solid supports including but not limited to nylon, rayon, and the like.

In accordance with a particular preferred embodiment, the pore size of the solid support is such that the probe, when bound to the analyte remains on the surface of the membrane **23**. Thus, for example, good results have been obtained with nitrocellulose having a pore size of from about 0.1 to 0.5 microns.

It is to be understood that the invention can be configured for detecting a broad range of analytes, including therapeutic drugs, drugs of abuse, hormones, vitamins, proteins (including antibodies of all classes), peptides, steroids, bacteria, viruses, parasites, components or products of bacteria, fungi, allergens of all types, antigens of all types, products or components of normal or malignant cells, and the like.

The following analytes are examples of analytes that may be tested using the present invention: T.sub.4, T.sub.3, digoxin, hCG, insulin, theophylline, luteinizing hormone, organisms causing or associated with various disease states, such as streptococcus pyogenes (group A), Herpes Simplex I and II, cytomegalovirus, chlamydiae, and others known in the art.

United States Patent No. 4,366,241 (Tom et al.) lists at columns 19-26 a variety of potential analytes of interest that are members of an immunologic pair, including proteins, blood clotting factors, hormones, microorganisms, pharmaceutical agents, and vitamins. Any of these analytes are suitable for use as the analyte in present invention.

Other examples of preferred ligands or analytes that may be detected include the following: human bone alkaline phosphatase antigen (HBAPAg); human chorionic gonadotropin (hCG); human luteinizing hormone (hLH); human follicle stimulating hormone (hFSH); creatine phosphokinase MB isoenzyme; ferritin; carcinoembryonic antigen (CEA); prostate specific antigen (PSA); CA-549 (a breast cancer antigen); hepatitis B surface antigen (HBsAg); hepatitis B surface antibody (HBsAb); hepatitis B core antigen (HBcAg); hepatitis B core antibody (HBcAb); hepatitis A virus antibody; an antigen of human immunodeficiency virus HIV I, such as gp 120, p66, p41, p31, p24 or p17; the p41 antigen of HIV II; and the respective antiligand (preferably a monoclonal antibody) to any one of the above ligands. The HIV antigens are described more fully in United States Pat. No. 5,120,662 and in Gelderblood et al., Virology 156: 171-176 1987, both of which are incorporated herein by reference.

As used herein, the term “probe-conjugate” refers to a species that is capable of carrying an analyte in a lateral flow assay to form a probe conjugate complex, which binds with a first capture reagent in the detection zone **24** to become a “sandwich complex” in detection area or detection zone **24**.

As used herein, the term "microparticle" is a more specific reference to a particular type of probe, and may include any beads or probes to which an antibody may be bound, whether covalently, or non-

covalently such as by adsorption. An additional requirement for some particles that are used in a quantitative assay is that the particle contributes a signal, usually light absorption, which would cause the zone in which the particles were located to have a different signal than the rest of the membrane **23**.

The microparticle employed typically must be capable of being retained by the membrane **23**. For example, when a microparticle is subject to liquid flow, the microparticle must be capable of remaining substantially immobilized. The microparticles may be of any shape but are preferably spherical. The nature of the microparticle may vary widely. It may be naturally occurring or synthetic. It can be a single material, a few materials, or a combination of a wide variety of materials. Naturally occurring microparticles include nuclei, mycoplasma, plasmids, plastids, mammalian cells (e.g., erythrocyte ghosts), unicellular microorganisms (e.g., bacteria) and the like. Synthetic microparticles may be prepared from synthetic or naturally occurring materials, or combinations thereof.

For example, latex microparticles may be prepared from a synthetic material such as styrene. Other microparticles may be prepared from naturally occurring materials, such as polysaccharides, e.g., agarose, or the like. (See, e.g., Gould, et al., U.S. Pat. No. 4,837,168, which describes the use of a variety of particles.) Preferred microparticles are microspheres of latex (i.e., a natural or a synthetic

polymer) or glass; more preferably microspheres of latex. The microspheres of glass or latex are also referred to in the art as "beads" or "microbeads."

A typical size for such beads is about 0.3 microns, but the invention may employ microparticles having greater or lesser size. For example, the mean diameter for the microparticle component of the present invention is within the range from about 0.01 microns to about 100 microns and more typically from about 0.1 microns to about 75 microns. The mean diameter and type of the microparticle chosen for a particular application will depend upon the pore size of the membrane and/or its composition.

Latex microparticles for use in the present invention are commercially available as polymeric microspheres of substantially uniform diameter (hereinafter "polymeric microspheres"), such as from Bangs Laboratories of Carmel, Indiana, or Dow Chemical Co. of Midland, Michigan. Although any polymeric microsphere that is capable of adsorbing or of being covalently bound to a binding partner may be used in the present invention, the polymeric microspheres typically are composed of one or more members of the group consisting of polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutyleneterephthalate, acrylonitrile,

vinylchloride-acrylates and the like or an aldehyde, carboxyl, amino, hydroxyl, or hydrazide derivative thereof.

The underivatized polymeric microspheres, such as polystyrene, are hydrophobic and passively adsorb other hydrophobic molecules, including most proteins and antibodies. Techniques for adsorbing a protein or polypeptide on a hydrophobic particle are provided in the publication by Cantarero, et al. "The Absorption Characteristics of Proteins for Polystyrene and Their Significance in Solid Phase Immunoassays," Analytical Biochemistry 105, 375-382 (1980); and Bangs, "Latex Immunoassays," J. Clin. Immunoassay, 13 127-131 (1980) both of which are incorporated herein by reference.

Various procedures for adsorbing molecules on polymeric microspheres are also described, in general terms, in Bangs, L. B., "Uniform Latex Particles," presented at a workshop at the 41st National Meeting, Amer. Assoc. Clin. Chem., 1989, and available in printed form from Seragen Diagnostics Inc., Indianapolis, Ind.; or Galloway, R. J., "Development of Microparticle Tests and Immunoassays, " i.e., Seradyn Inc. of Indiana which is incorporated herein by reference.

The covalent bonding of a binding partner to a microparticle may be accomplished either directly, such as by reacting an activated chemical functional group on the surface of a microparticle with an appropriate chemical functional group on the binding partner, or indirectly, such as by covalently binding the binding partner to a spacer

molecule that has been covalently bound to the surface of the microparticle.

By the phrase "membrane" as used herein is meant a test device that employs a membrane and one or more reagents to detect the concentration of an analyte of interest in a test solution, preferably an aqueous test solution. At least one of the reagents associated with the membrane device is a binding partner of the analyte of interest.

The calibration device and method of the present invention is useful with essentially any membrane-based devices. A particularly preferred use for the calibrator of the present invention is as an internal calibrator. The choice and size of a microparticle for the stabilized internal calibrator of a membrane-based device is influenced by the choice of material for the membrane. The internal calibrator of the present invention may be affixed to the membrane by covalent or non-covalent bonding.

In the practice of the invention, calibration and sample testing may be conducted under essentially exactly the same conditions at the same time, thus providing reliable quantitative results, with increased sensitivity.

The invention also may be employed for semi-quantitative detection. As the multiple control lines provide a range of signal intensities, the signal intensity of a given detection line can be compared (i.e. such as for example, visually) with the intensity of the control lines.

Based upon the intensity range wherein the detection line falls, the possible concentration range for the analyte may be determined. The probes may be latex beads labeled with any signal generating species or the labeled latex beads further conjugated with antibodies.

The signal ratio between the detection lines and the control lines may be plotted against the analyte concentrations for a range of analyte concentrations to generate a calibration curve, such as shown in Figure **2** herein. To determine the quantity of an unknown sample, the signal ratio may be converted to analyte concentration according to the calibration curve.

Example 1

Polyethyleneimine was used to demonstrate the invention. A 7.4% polyethyleneimine aqueous solution (stock solution)(1x), a 10x dilution and a 100x dilution were stripped onto Millipore SX membrane to form three control lines. The membrane was dried for about 1 hour at about 37 degrees Centigrade. A wicking pad was attached upon one end of the membrane. The other end of the membrane was inserted into a suspension of blue latex beads or red fluorescent latex beads containing 1.6% Tween 20 (a surfactant) or antibody-conjugated latex beads with 1.6% Tween 20. Five minutes later, the beads were captured on the lines where the polyethyleneimine solution was stripped.

Example 2

In another example, the membrane was stripped with three different polyethyleneimine solutions (1x, 10x, 100x dilution) on the lines of the calibration zone **32** and anti C-reactive protein (CRP) monoclonal antibody (Mab A5804) was immobilized on the detection zone. The membrane was dried for about one hour at about 37 degrees Centigrade, and the wicking pad was attached to the end of the membrane to form a half dipstick. The other end of the half dipstick was inserted into a solution with CRP antigen and anti CRP monoclonal antibody (Mab A58011) conjugated to latex particles (blue). The solution flowed through the detection and control zones, and then to the wicking pad. One blue line on the detection zone and three blue lines on the control zone were observed.

In the above examples, it was observed that the signal intensities of the control lines were significantly different. The control line stripped with 7.4% polyethyleneimine stock solution exhibited the most signal intensity while the control line stripped with 100x dilution solution exhibited the least signal intensity. This observation was true for blue colored and red fluorescent latex beads alone, as well as these beads further conjugated with antibodies.

Example 3

The membrane HF 09002 was stripped with 0.14% (calibration #1), 0.64% (calibration #2) and 1.4% (calibration #3) of polyethyleneimine solution on the calibration or calibration zone **32**. On

the detection line **24**, anti CRP monoclonal antibody at 1 mg/ml (Mab A5804) was immobilized. The membrane was dried at 37 C for one hour and the wicking pad **28** was attached to the end of the membrane to form the half stick. The half sticks were inserted into the solutions containing the following nano-grams of CRP antigen (0. 0.54, 5.4 and 54) with excess amount of blue latex beads which are conjugated with anti CRP monoclonal antibody (Mab58011). It was observed that three calibration lines were formed with different intensities, where the line had 1.4% polyethyleneimine concentration exhibits the highest line intensity and the line had 0.14% polyethyleneimine concentration had the least line intensity. The same experiments were carried out with a mixture of blue latex beads and latex beads antibody conjugate and the same results were observed. (Experiments with different polyelectrolytes were also carried out, such that line 1, 2 and 3 may be totally different polymers).

Results from Example **3** are provided below in Table **1**.

Table 1: Signal Intensities of Calibration and Detection Lines

Calibration #1	A	A	A	A
Calibration #2	B	B	B	B
Calibration #3	C	C	C	C
Detection Line	None (0 ng)	C (0.54 ng)	B (5.4 ng)	A (54 ng)

The results indicated that the intensity of calibration line #3 represents 0.54 ng of analyte, calibration line #2 represents 5.4 ng of analyte, and calibration line #1 represents 54 ng of analyte. When an unknown sample was tested, the analyte concentration could be visually determined by comparing the detection line intensity with the three calibration lines.

When the detection line intensity was less than calibration line #3, the concentration of the analyte was determined to be less than about 0.54 ng. When the detection line intensity was visually determined to be between calibration line #3 and #2, the analyte concentration was found to be between 0.54 and 5.4 ng. When the detection line intensity was found to be higher than calibration line #3, the analyte concentration was determined to be higher than 54 ng.

Example 4

The same procedure as Example **3** was conducted, with the exception that the concentration of the analyte was quantified by a electronic reading device. In such diagnostic reading devices, electronic routines make it possible to read automatically the intensities of calibration and detection lines and provide a readout or display for the analyte concentration.

The latex beads generate a detectable colored light signal, from both the detection zone **31** and the control lines of the calibration zone **32**. The reading device provides a comparison means for comparing the

intensity of colored light signals generated by latex beads positioned upon the control lines **25-27** with the intensity of signals generated by microparticle-analyte conjugates positioned upon the detection zone **31**.

Example 5

In yet another application of the invention, it is possible to use fluorescence to determine the amount of analyte in a test sample. In this manner, it is possible to use a probe or a microparticle which itself is capable of exhibiting the property of fluorescence, in which signals are generated from the probe or microparticle once it has been deposited in either the calibration zone **32** or the detection zone **31**. A receiver or a receiving device is capable of measuring the amount of signal generated in the detection zone **31** and the calibration zone **32**, and making the appropriate comparisons to determine the quantity of analyte in a given test sample.

It is understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention, which broader aspects are embodied in the exemplary constructions. The invention is shown by example in the appended claims.